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DEVELOPMENT OF DNA MARKERS FOR WHEAT QUALITY TRAITS IN CPS WHITE WHEAT USING QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS

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Development of DNA Markers for Wheat Quality Traits in CPS White Wheat Using Quantitative Trait Loci (QTL) Analysis

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by

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Abstract

This project has aimed to develop DNA markers for wheat quality traits that can be applied in wheat breeding programs. The doubled haploid population AC Karma/SC8021V2, consisting of 111 lines, along with 10 checks was grown at three locations in Saskatchewan and one location in Manitoba for the years 1999-2001. Data on 7 agronomic traits and 67 quality traits was collected and summarized. A rudimentary molecular genetic map consisting of 73 markers has been produced and some preliminary associations with quality traits have been made. An addendum to this report is proposed when the map and association of the map with quality traits has been completed.

Executive Summary

This reports provides the methods used to collect the end-use quality data and summarizes all of the data collected for this project.

The agronomic data collected in this project included yield, maturity, lodging, height, test weight, kernel weight and common bunt resistance. Some leaf rust data based on inoculating with a race mixture was collected, but is not presented here as it will have little value in determining the genetics of resistance to leaf rust. Since single-row plots were used in this study, yield estimations are not expected to be very good; however, having 10 locations with 4 replications per location may provide that are adequate. Maturity and lodging were compromised at the Saskatchewan locations in 2001 due to droughty conditions.

The quality data collected in this project is quite extensive and comprehensive. Residual grain and flour will allow for additional tests to be considered. Whole grain protein and polyphenol oxidase activity was determined from 10 environments (location-years). Meal color, SDS-sedimentation volume and falling number data were collected from 10 environments. Grain hardness as measured using an NIR PSI calibration was measured from 6 environments, while whole grain SKCS (single kernel characterization system) measures were collected from 3 environments. Flour milling was carried out on samples from 9 environments. Ash content, flour color, mixograph, farinograph, amylograph, noodle and baking quality were determined on samples from 6 to 9 environments. Analysis of variance (ANOVA) was conducted to summarize data for each year and over the entire project. Coefficient of determination (R^2) from ANOVA for each trait is reported for the combined years analysis and ranged for $R^2 = 0.27$ to $R^2 = 0.95$. Correlations among the characteristics demonstrated a number of associations that have been reported in previously in the literature. These observations serve to demonstrate that the data was collected in an appropriate manner and that genetic associations that may be found later will be of value.

Further development of the genetic map of AC Karma/SC8021V2 at ECORC in Ottawa has been stopped due to personnel moving on to other positions and inadequate funding. Recently, a new Matching Investment Initiative project within Agriculture and Agri-food Canada has been approved. Part of this project includes the development of a molecular genetic map for AC Karma/SC8021V2. This can be expected within the next two years.

I propose to provide the Saskatchewan Agricultural Development Fund with an updated final report when the map and associated analysis has been completed.

Technical Report

Methods

Field replications for each location were combined to provide a larger composite sample size for each line for further quality analysis. The composite consisted of 220 g from each replication giving a total of 660 g for each line per location. Three sets of composite samples were prepared for each line: 10 g composite for grain hardness tests, 50 g composite for all meal tests and the PPO test, and a 600 g sample for milling and flour tests. Sample size did vary so some of the composites were not made up of equal amounts from each repetition.

A) Tests on Whole Grain

The **kernel weight** (KWT) was obtained using an automatic (Agriculex Inc.) seed counter that counted out 200 kernels. The 200-kernel sample was weighed and multiplied by 5/1000 to give the KWT in milligrams. **Test weight** (TWT) was determined for each sample (rep and location) using a 1/4 L test weight container.

Polyphenol oxidase (PPO) activity in wheat has been related to discolouration of Asian noodles during storage. The method used to determine PPO activity was a modification of the method developed by Morris et al (1998). A 50mM solution of MOPS (3-[N-Morpholino] propane sulfonic acid) buffer was prepared by weighing 10.5 g MOPS into a 1 L cylinder, and adding distilled water to 1 L. A 5 mM L-DOPA (L-3,4-Dihydroxyphenyl-Alanine) solution in 50 mM MOPS buffer was prepared by adding 1.0 g L-DOPA to one litre of MOPS buffer. Solutions were prepared daily. Twenty seeds were randomly selected from each sample, placed in 15 mL centrifuge tubes, 6 mL of L-DOPA/MOPS solution pipetted into each tube and the lids closed. One blank tube was prepared containing only the solution and two check samples (high/low PPO) were included. The tubes were placed in a tray constructed to hold 24 tubes and inserted into a SDS-sedimentation shaker. After gentle shaking for 30 minutes, 200µ were pipetted from each tube into the 24 wells of a microtitre plate. The optical density of each sample was determined at 490nm using a microplate reader (Microplate Manager Bio-Rad Laboratories, Inc.)

B) Tests on Whole Meal

The 50 gram samples were ground into meal on the UDY Corporation cyclone mill. The 1 mm screen was used and the ground sample was stored in sealed polyethylene bags.

Grain protein content (%N x 5.7) was determined using a nitrogen combustion analyzer (FP-428, Leco Corp., St. Joseph, MI).

Meal color was measured using a HunterLab Colorquest spectrocolorimeter (Hunter Associates Laboratory, Reston, Virginia). Three color parameters (DT65/10°) of the meal were recorded:

L = higher values indicated whiter material, lower values indicated darker material
a = higher values indicated redder material, lower values indicated greener material
b = higher values indicated yellower material, lower values indicated bluer material

Standardization and calibration procedures were performed daily, in the morning and after lunch. Ground sample was placed in a petri dish to a depth of approximately 0.5cm. The meal was packed down lightly, placed over the light source, and the Lab values recorded.

Particle size index (PSI) was measured to give an indication of grain hardness by Near Infrared Reflectance (NIR) using a Bran+Luebbe InfraAnalyzer 360. NIR measures the relative intensity of near infrared reflected light absorbed at the surface of the sample at different wavelengths and uses these measurements to compute composition or other quality parameters (Bran+Luebbe NIR450 Operation Manual, 1993). A calibration for PSI was previously developed for the NIR360 based on the PSI method of Williams and Sobering (1986), which defines PSI as the percent of the ground material which passes through a 200-mesh screen (75μm). A lower PSI value indicates larger particle size, and therefore harder grain. Before reading the test samples, a standard sample and a ceramic tile were checked to ensure consistent readings. The test sample was poured into the NIR cell, gently patted down, and levelled off. The lid was put on the cell, placed in the NIR drawer, and the values were recorded.

The **Falling Number** (AACC method 56-81B, 1995) system gives an indication of alpha-amylase activity in a grain sample. The methods is based on the rapid gelatinization of a meal/water suspension in a boiling water bath and the subsequent liquefaction of the gelatinized starch by the alpha-amylase in the sample. The falling number is the total time in seconds from the immersion of the viscometer tubes into the water bath until the viscometer stirrer has dropped down through the gelatinized suspension, and includes the 55second initial stirring phase (Falling Number 1400 Operation Manual, 1987). The falling number apparatus was turned on, water level checked and adjusted, and cooling water line turned on. When the apparatus reached boiling point, a 7g sample of ground meal was added to 25mL of distilled water in the falling number tube. The tube was stoppered and shaken 15-20 times, until well suspended. The sides of the tube was wiped down with the stirrer, the tube and stirrer placed into the water bath and the machine turned on. When the stirring rod had dropped the full distance through the gelatinized suspension the value was recorded in seconds and the next sample was prepared.

The **Sodium Dodecyl Sulfate (SDS) Sedimentation** test is a wheat quality screening test which has been related to gluten strength and baking quality. AACC method 56-70 (1995) was used, with some modification. A 2% SDS-lactic acid solution was prepared by dissolving 80g SDS in 4L of distilled water and adding 80mL of 2% lactic acid. Large bottles were filled: one with distilled water and one with the 2% SDS solution. Automatic dispensers were set to deliver 50mL of each solution. Eight samples were tested at a time. Eight weighed samples of 5g meal were quickly added to 8-100mL graduated cylinders. 50mL of distilled water was dispensed into the first graduated cylinder, the cylinder stoppered, the timer started, and the cylinder vigorously hand shaken 20 times before being placed on the shaker. The remaining seven samples were prepared in the same manner at 20sec intervals. At 4 minutes, 50mL of SDS solution was added to the first tube and placed back on the shaker. The remaining tubes were filled with SDS solution at 20sec intervals and placed on the shaker. At 10 minutes the first cylinder was removed from the shaker and left to settle on the counter top. The remaining cylinders were removed and set on the counter at 20sec intervals. At 30 minutes, the sedimentation volume (mL) of the first tube was recorded and the remaining sample read at 20sec intervals.

C) Tests on Flour

Prior to **flour milling**, **moisture content** of the grain samples was determined. A random selection of 10-15 samples from each location was selected. A10g subsample taken of each of these and ground on a Thomas-Wiley Intermediate mill (Thomas Scientific, Swedesboro, NJ) in order to minimize moisture loss on grinding. The moisture was determined using a standard

oven drying procedure of 1 hour at 130°C (AACC method 44-15A, 1994). The average moisture content of samples from each location was calculated and assumed to represent grain moisture of samples from that site. 600g grain samples were tempered to 14.5% the day prior to milling. The Brabender Quadromat Jr. mill was used to mill the grain into flour (AACC method 26-50, 1995). The machine was first warmed up for ten minutes with the 6XX (235μm opening) reel sifter in place, then the airflow and feed rate settings were checked. A check sample was run through the machine and the flour yield determined prior to milling test samples. **Flour yield** for all the samples was calculated as:

(flour yield/total yeild)*100, where total yeild = flour + bran

Flour color and **flour protein content** were measured using the same procedure as for meal.

Flour **ash content** was determined according to AACC Method 08-01 (1995), with some modification. A 3g sample of flour was weighed into porcelain crucibles and burned for 4 hours at 600°C. After cooling in a dessicator, the unburned material remaining (ash) was weighed and reported as the percentage of the original flour weight.

The **mixograph** was used to evaluate mixing properties of the flour samples. A 10 g mixograph (National Manufacturing Co.) was used in conjunction with the Mixsmart computer program (National Cereal Chemistry Equipment, Version 3.40, 1999), according to AACC method 54-40A (1995). A 10 g flour sample (as is mb) was measured into the mixing bowl and 6.5 mL of water (65% absorption) added. The bowl was locked into position, the upper pins lowered into the bowl and the mixograph was run for 7 minutes. The parameters recorded were: mixing development time (MDT), peak height (PKH), slope after peak (SAP), band width at 6 minutes (BW6), band width at peak (PBW), energy to peak (ETP) and total energy under the curve(TEG).

The **farinograph** (C.W. Brabender Instruments, Inc., AACC method 54-21, 1995) was used to assess dough mixing characteristics and water absorption capacity of the flour samples. The curves were read manually and the parameters recorded were: dough development time (DDT, the time it takes for the dough to reach peak development), farinograph absorption (FAB, the amount of water required to centre the curve at peak on the 500BU line), mixing tolerance index (MTI, the drop in the top of the curve 5min after peak), and stability (STA, the difference in time from when the top of the curve reaches and departs from the 500BU line). The water bath (30°C) was turned on and the farinograph allowed to run empty for 10-15min. Prior to starting the first test, the recording arm was checked to make sure it corresponded with the center of the recording paper. Fifty grams of flour (as is mb) were measured into the mixing bowl and the lid closed. An estimated amount of distilled water was added to the flour sample from the buret, and the mixer and chart paper started. If the peak of the curve was not centered on the 500 BU line, the machine was stopped, and the amount of water required to center the curve was reevaluated. After cleaning the bowl and mixing blades, a second sample of another 50g of flour was added and retested.

The **micro-visco-amylograph** (C.W. Brabender Instruments, Inc.) was used to assess the starch pasting properties of the test samples. A slurry was prepared by mixing 8g of flour with 100mL distilled water in a glass beaker and mixing with a plastic spatula until smooth. The slurry was poured into the bowl, which was then attached to the machine with the mixing paddle in place. A standard pasting curve was obtained with the following protocol: mixing speed 240 (1/min), measuring range 75cmg, heating/cooling rate 7.5°C/min, start temperature 30°C, maximum temperature 95°C, upper hold time 5min, end temperature 50°C and final hold time 1 minute. The test run took approximately 20 minutes to complete. The data derived from the test were: Peak viscosity, viscosity at the beginning and end of the upper holding temperature,

breakdown (drop in viscosity curve during upper hold time) and setback (increase in viscosity during the cooling phase).

The **Optimized Straight-Dough Bread-Making Method** (AACC Method 10-10B, 1995) was used to assess the baking potential of the test lines.

The formulation was as follows:

Flour (as is)	100.0 g
Yeast (compressed)	2.0% (approx 1% active dry yeast)
Salt	1.5%
Sugar	6.0%
Shortening	3.0%
Ammonium phosphate	0.1%
Malt powder (60°L)	0.2%
Whey powder	2.0%
Ascorbic Acid	25 ppm
Distilled water	FAB-3%

On the day before baking, all dry ingredients (flour, malt powder, whey powder) and shortening were weighed into numbered and lidded cans. The first two bakes were "blanks" (bulk flour), followed by a control flour of known baking quality. The day of baking, all equipment was turned on and allowed to warm up for at least 30 minutes while solutions were prepared. Equipment used were: GRL-200 mixer (135rpm) and water bath (30°C), linear chart recorder set at 1cm/min, fermentation cabinet (30°C and 85% RH), reel oven (400°F), roller-sheeter apparatus and rapeseed displacement volumeter. The salt/sugar/ammonium phosphate solution and the yeast solution were prepared to deliver the appropriate amount of each ingredient in 25mL aliquots. Ascorbic acid solution was prepared to deliver 25ppm in 1mL solution. Dough water was calculated to take into account the amount of water added in solution. All ingredients were mixed until 1min past peak dough development, according to a mixing curve. After mixing, the dough was rounded by hand 7 times, placed in a covered fermentation bowl, and set in the fermentation cabinet. The time was recorded on the bake sheet and the next samples were prepared in the same manner. After a fermentation period of 105 minutes, the dough was punched by passing it through the sheeter (3/16inch gap) once, rounded and returned to the fermentation cabinet. After a further 50 minutes, the punching procedure was repeated. After a final fermentation period of 25 minutes, the dough was sheeted by passing it through the sheeter 3 times, at progressively smaller gap settings (5/16 in, 3/16 in, and 1/8 in). The sheeted dough was rolled into a cylinder, placed in the roller for 30sec, applying moderate force, and placed in a greased pup-loaf pan. The made-up dough was proofed for 1hour in the fermentation cabinet and then baked for 25 minutes. After cooling 30 minutes, loaf volume was measured and external/internal loaf characteristics were evaluated subjectively.

White salted noodles were prepared using a small-scale method developed by G.H. Zheng (Dept. of Plant Sciences, University of Saskatchewan, 1999). A 7.5 % salt solution was prepared (7.5g NaCl/100mL distilled water). Ten grams of flour was weighed out and placed in a plastic container and 4mLof salt solution was added (40% absorption, 3%NaCl). The flour-salt solution mixture was mixed with a plastic spatula until no water was visible (10-20sec), transferred to a coffee grinder, and mixed for a further 10sec. The sample was then transferred to a plastic container, covered and left to rest for 15 minutes at room temperature. After resting the sample was pressed between two metal mini bread pans, removed and passed through setting #2 of a small pasta sheeter three times. The sheet was folded in half after each pass through the

rolls. The dough sheet was then sealed in a polyethylene bag and left in darkness at room temperature for 30 minutes. The dough sheet was then sheeted by successive passes through #2,#3,#4,#5,and #6 roll gaps, always in the same direction. Six 3cm diameter circles were cut from the dough sheet using the plastic cutter.

Two of the noodles were placed in sealed polyethylene bags and used for raw noodle color evaluation. The Miniscan XE Colorimeter (Hunter Associates Laboratory, Reston, Virginia) was used to obtain L, a, b and YI (yellow index) values (DT65/10°) of the raw noodles at 0, 2 and 24 hours. The remaining 4 noodles were placed in gently in boiling water for 5 minutes. The cooked noodles were transferred to a sieve and place in a running tap water bath for 1 min. After gently removing surplus water using paper towels, two of the cooked noodle were placed in sealed polyethylene and Lab values were measured at 0, 2 and 24 hours. The remaining 2 cooked noodles were evaluated immediately for texture using the TMS-90 Texture Test System (Food Technology Corp., Rockville, Maryland). A standard TPA (texture profile analysis) curve was generated for each noodle sample (2reps/test sample) using the TPA-1 cell. Parameters obtained were: hardness, cohesiveness, adhesiveness, adhesive force, springiness, stringiness, gumminess and chewiness.

Summary of agronomic data 1999-2001

This experiment was grown at 4 locations each year: one in Manitoba and three in Saskatchewan. The Glenlea site experienced difficult harvest conditions in 1999 leading to many samples being contaminated with soil. The Glenlea 2000 site was poorly looked after and no useful data was obtained. The Swift Current 2000 location was inadvertently discarded after harvest, which was unfortunate. The 2001 location at Swift Current suffered from drought, which reduced seed yields. Sufficient seed was produced to do whole seed and meal tests but no tests involving flour. The Kernen site was switched to Goodale for 2000 and 2001 as the latter location could be planted earlier. This was important as some lines from this population showed a requirement for vernalization. The Seed Farm (Saskatoon) and Goodale locations were the most successful for this study.

Yield data was collected from 10 site-years. Height data was collected from 5 site-years. Test weight and kernel weight data was collected from 9 site-years. Maturity was collected from 6 site years and lodging resistance data collected from 3 site years. Environments suitable for collecting useful lodging data were not experienced in Saskatchewan in 2000-2001. All agronomic data is reported in Tables 1-7 of Appendix A.

Summary of quality data

All quality data were collected using a composite sample developed from the field experiments for each location that was grown. Using each location-year as an environment, an analysis of variance was conducted for each trait. The number of environments and the amount of variation explained by each analysis of variance (R^2) is shown in Tables 1-4. The number of environments sampled was determined by the amount of time and/or effort required to collect the data and the suitability of the environment. Several environments were not used because the grain produced was not sound. For example, Kernen (1999) was not used for noodle tests since the field experiment suffered frost damage, resulting in a lot of immature green seeds that interfere with noodle colour measurements. Noodle making and texture profile analysis (TPA) of the noodles were the most time consuming operations conducted in this project, so only two locations per year were evaluated.

All quality data is summarized in 66 tables in Appendix B. A correlation matrix amongst all of these traits has been developed. Selected portions of the matrix will be shown as appropriate. Only highly significant ($p \leq 0.01$) correlations will be discussed.

Table 1. Traits with 70 to 90 percent of variation explained by analysis of variance using the combined dataset and number of locations included in the analysis

Trait	R^2	Number of environments
Microamylograph viscosity at end	0.95	6
Particle size index (PSI)	0.87	6
Falling number (FN)	0.79	10
Sodium dodecyl sulfate sedimentation	0.79	10
Whole meal L-value	0.72	10

Table 2. Traits with 50 to 70 percent of variation explained by analysis of variance using the combined dataset and number of locations included in the analysis

Trait	R^2	# env	Trait	R^2	# env
Microamylograph viscosity setback	0.67	6	Raw noodle yellow index at 0 hours	0.53	6
Microamylograph viscosity peak	0.64	6	Flour a-value	0.53	8
Whole meal b-value	0.63	10	Farinograph water absorption	0.53	7
Polyphenol oxidase absorbance 435 nm	0.62	10	Mixograph total energy	0.53	9
Raw noodle L-value at 24 hours	0.60	6	Whole meal a-value	0.52	10
Mixograph peak height	0.59	9	Flour protein (LECO)	0.52	7
Farinograph mixing tolerance index	0.58	7	Raw noodle a-value at 24 hours	0.51	6
Flour L-value	0.57	8	Cooked noodle yellow index at 24 hours	0.51	6
Microamylograph viscosity at start	0.56	6	Raw noodle b-value at 24 hours	0.50	6
TPA springiness	0.55	5	Cooked noodle yellow index at 0 hours	0.50	6
Flour yield	0.55	8	Raw noodle yellow index at 2 hours	0.50	6
Raw noodle L-value at 0 hours	0.55	6	Microamylograph viscosity breakdown	0.50	6
Cooked noodle b-value at 24 hours	0.54	6			

Table 3. Traits with 40 to 50 percent of variation explained by analysis of variance using the combined dataset and number of locations included in the analysis

Trait	R^2	# env	Trait	R^2	# env
Raw noodle a-value at 0 hours	0.49	6	TPA adhesiveness	0.46	5
Raw noodle yellow index at 24 hours	0.49	6	Flour ash	0.45	7
Raw noodle b-value at 2 hours	0.48	6	Cooked noodle b-value at 0 hours	0.44	6
Mixograph slope after peak	0.48	9	TPA hardness	0.44	5
Cooked noodle b-value at 2 hours	0.48	6	Flour b-value	0.43	8
Cooked noodle yellow index at 2 hours	0.47	6	TPA stringiness	0.42	5
Raw noodle a-value at 2 hours	0.47	6	Grain protein (LECO)	0.42	10
Farinograph stability	0.47	7	Farinograph dough development time	0.41	7
Raw noodle L-value at 2 hours	0.46	6	CSP mixing time	0.41	7

Table 4. Traits with 30 to 40 percent of variation explained by analysis of variance using the combined dataset and number of locations included in the analysis

Trait	R^2	# env	Trait	R^2	# env
Raw noodle b-value at 0 hours	0.39	6	Loaf crumb colour	0.35	5
Loaf crumb structure	0.39	5	Cooked noodle a-value at 0 hours	0.33	6
Cooked L-value 0 hours	0.38	6	Cooked noodle a-value at 2 hours	0.33	6
Cooked L-value at 2 hours	0.37	6	Mixograph bandwidth energy	0.32	9
Mixograph mixing development time	0.37	9	Cooked noodle a-value at 24 hours	0.31	6
Loaf volume	0.37	7	Loaf crust colour	0.31	5
TPA chewyness	0.37	5	Cooked noodle L-value at 24 hours	0.31	6
Mixograph bandwidth 6 min after peak	0.36	9	TPA cohesiveness	0.28	5
Loaf appearance	0.36	7	TPA gumminess	0.27	5
TPA adhesive force	0.36	5			

Predictive tests

Although not a primary goal of this project, a number of high correlations for yellowness were observed (>0.80) that would allow for effective indirect selection to occur. In Table 5, it is evident that b-values (yellowness) measured on flour were strongly correlated with b-values and yellow index of raw and cooked noodles. Thus, the yellowness of the flour is predictive of yellowness in a product made from the flour.

The more common situation was correlations less than 0.80. Although correlations in this project were highly significant down to r = 0.234, these low correlations do not equate to effective indirect selection. Loaf volume as measured by baking a pup loaf, for example, showed significant correlations for several mixograph and farinograph parameters but all in the r = 0.3-0.4 range. As a result, these three procedures are often done together as no single measure adequately predicts the loaf volume produced by a specific genotype.

Starch pasting characteristics

The experimental procedures used in this project feature the evaluation of features of wheat protein, starch and the interaction of these two components. Falling number and starch pasting characteristics as measured by the microamylograph were the only evaluations of starch characteristics used in this study. All of the characteristics of starch pasting were primarily correlated only with falling number in this study: there were no significant correlations to dough mixing characteristics as evaluated by mixograph and farinograph and there were no correlations to noodle colour or texture characteristics. Falling numbers showed significant correlations to mixograph peak height, bandwidth energy and total energy and also farinograph flour water absorption and mixing tolerance index.

Table 5. Correlations among Hunterlab L, a, b colour values measured on whole meal, flour, and raw and cooked noodles evaluated at 0, 2 and 24 hours after sheeting or cooking

	MEAL_L	MEAL_A	MEAL_B	FLOUR_L	FLOUR_A	FLOUR_B	RL0	RL2	RL24	RA0	RA2	RA24	RB0	RB2	RB24	RY0	RY2	RY24	CL0	CL2	CL24	CA0	CA2	CA24	CB0	CB2	CB24	CY0	CY2	CY24
MEAL_L	1.00																													
MEAL_A	-0.91	1.00																												
MEAL_B	-0.72	0.58	1.00																											
FLOUR_L	0.93	-0.82	-0.83	1.00																										
FLOUR_A	-0.88	0.91	0.60	-0.89	1.00																									
FLOUR_B	-0.59	0.48	0.93	-0.70	0.50	1.00																								
RL0	0.68	-0.56	-0.60	0.69	-0.56	-0.57	1.00																							
RL2	0.67	-0.55	-0.59	0.68	-0.54	-0.56	0.95	1.00																						
RL24	0.47	-0.32	-0.41	0.44	-0.29	-0.38	0.73	0.78	1.00																					
RA0	-0.72	0.66	0.57	-0.69	0.66	0.55	-0.92	-0.92	-0.67	1.00																				
RA2	-0.71	0.65	0.54	-0.68	0.64	0.52	-0.89	-0.94	-0.68	0.97	1.00																			
RA24	-0.67	0.61	0.48	-0.63	0.58	0.43	-0.86	-0.91	-0.74	0.92	0.94	1.00																		
RB0	-0.07	0.01	0.52	-0.17	-0.01	0.68	-0.37	-0.34	-0.22	0.33	0.29	0.22	1.00																	
RB2	-0.13	0.05	0.60	-0.23	0.04	0.77	-0.45	-0.45	-0.30	0.42	0.40	0.30	0.87	1.00																
RB24	-0.06	0.01	0.53	-0.15	-0.02	0.68	-0.34	-0.33	-0.08	0.33	0.32	0.25	0.84	0.93	1.00															
RY0	-0.27	0.18	0.67	-0.36	0.17	0.81	-0.61	-0.57	-0.42	0.56	0.52	0.44	0.67	0.96	0.88	1.00														
RY2	-0.30	0.20	0.66	-0.37	0.18	0.80	-0.63	-0.64	-0.46	0.60	0.60	0.50	0.83	0.97	0.88	0.97	1.00													
RY24	-0.28	0.18	0.64	-0.34	0.14	0.75	-0.63	-0.64	-0.47	0.61	0.60	0.56	0.81	0.93	0.91	0.94	0.96	1.00												
CL0	0.64	-0.57	-0.57	0.63	-0.52	-0.58	0.86	0.90	0.60	-0.88	-0.89	-0.83	-0.36	-0.47	-0.40	-0.58	-0.64	-0.64	1.00											
CL2	0.59	-0.53	-0.51	0.57	-0.49	-0.52	0.83	0.87	0.59	-0.86	-0.88	-0.82	-0.35	-0.47	-0.40	-0.56	-0.63	-0.63	0.96	1.00										
CL24	0.57	-0.53	-0.48	0.54	-0.48	-0.48	0.71	0.74	0.49	-0.76	-0.77	-0.71	-0.28	-0.39	-0.33	-0.47	-0.53	-0.53	0.88	0.93	1.00									
CA0	-0.68	0.69	0.39	-0.62	0.65	0.32	-0.65	-0.67	-0.46	0.74	0.74	0.71	0.01	0.07	0.01	0.20	0.24	0.25	-0.80	-0.83	-0.85	1.00								
CA2	-0.66	0.66	0.38	-0.61	0.63	0.32	-0.68	-0.70	-0.48	0.75	0.77	0.74	0.03	0.11	0.04	0.23	0.28	0.28	-0.81	-0.87	-0.88	0.96	1.00							
CA24	-0.67	0.66	0.40	-0.60	0.63	0.35	-0.65	-0.67	-0.44	0.72	0.74	0.70	0.06	0.15	0.09	0.26	0.31	0.31	-0.78	-0.8[illegible]	-0.89	0.92	0.93	1.00						
CB0	-0.21	0.13	0.59	-0.27	0.10	0.73	-0.52	-0.54	-0.34	0.54	0.52	0.43	0.91	0.90	0.87	0.90	0.91	0.91	-0.63	-0.62	-0.56	0.28	0.29	0.31	1.00					
CB2	-0.21	0.13	0.59	-0.28	0.11	0.75	-0.52	-0.53	-0.35	0.53	0.51	0.40	0.78	0.94	0.88	0.93	0.94	0.91	-0.64	-0.65	-0.59	0.29	0.33	0.35	0.92	1.00				
CB24	-0.20	0.13	0.58	-0.27	0.10	0.73	-0.53	-0.54	-0.36	0.54	0.52	0.42	0.81	0.94	0.88	0.92	0.94	0.92	-0.63	-0.65	-0.60	0.29	0.31	0.35	0.93	0.97	1.00			
CY0	-0.32	0.24	0.64	-0.37	0.19	0.78	-0.62	-0.64	-0.43	0.64	0.62	0.53	0.78	0.91	0.85	0.92	0.95	0.93	-0.74	-0.74	-0.67	0.40	0.42	0.44	0.85	0.97	0.97	1.00		
CY2	-0.27	0.20	0.60	-0.32	0.16	0.74	-0.58	-0.60	-0.39	0.59	0.58	0.48	0.77	0.90	0.84	0.90	0.93	0.91	-0.72	-0.74	-0.68	0.40	0.44	0.45	0.93	0.97	0.97	0.98	1.00	
CY24	-0.28	0.21	0.60	-0.34	0.18	0.74	-0.60	-0.61	-0.41	0.62	0.60	0.50	0.77	0.90	0.84	0.91	0.93	0.91	-0.72	-0.74	-0.71	0.41	0.44	0.48	0.92	0.97	0.99	0.97	0.98	1.00

Table 5. Correlations among Hunterlab L, a, b colour values measured on whole meal, flour, and raw and cooked noodles evaluated at 0, 2 and 24 hours after sheeting or cooking

	MEAL_L	MEAL_A	MEAL_B	FLOUR_L	FLOUR_A	FLOUR_B	RL0	RL2	RL24	RA0	RA2	RA24	RB0	RB2	RB24	RY0	RY2	RY24	CL0	CL2	CL24	CA0	CA2	CA24	CB0	CB2	CB24	CY0	CY2	CY24
MEAL_L	1.00																													
MEAL_A	-0.91	1.00																												
MEAL_B	-0.72	0.58	1.00																											
FLOUR_L	0.93	-0.82	-0.83	1.00																										
FLOUR_A	-0.88	0.91	0.60	-0.89	1.00																									
FLOUR_B	-0.59	0.48	0.93	-0.70	0.50	1.00																								
RL0	0.69	-0.56	-0.60	0.69	-0.56	-0.57	1.00																							
RL2	0.67	-0.50	-0.59	0.68	-0.54	-0.56	0.95	1.00																						
RL24	0.47	-0.32	-0.41	0.44	-0.29	-0.38	0.73	0.78	1.00																					
RA0	-0.72	0.60	0.57	-0.69	0.66	0.55	-0.92	-0.92	-0.67	1.00																				
RA2	-0.71	0.65	0.54	-0.68	0.64	0.52	-0.89	-0.93	-0.68	0.97	1.00																			
RA24	-0.67	0.61	0.48	-0.63	0.58	0.43	-0.86	-0.91	-0.74	0.92	0.94	1.00																		
RB0	-0.07	0.01	0.62	-0.17	-0.01	0.68	-0.37	-0.34	-0.22	0.33	0.29	0.22	1.00																	
RB2	-0.13	0.05	0.60	-0.23	0.04	0.77	-0.45	-0.55	-0.30	0.42	0.40	0.30	0.87	1.00																
RB24	-0.06	0.01	0.53	-0.15	-0.02	0.68	-0.34	-0.33	-0.08	0.33	0.32	0.26	0.84	0.93	1.00															
RY0	-0.27	0.18	0.67	-0.36	0.17	0.81	-0.61	-0.57	-0.42	0.56	0.52	0.44	0.87	0.96	0.88	1.00														
RY2	-0.30	0.20	0.66	-0.37	0.18	0.80	-0.63	-0.64	-0.46	0.60	0.60	0.50	0.83	0.97	0.88	0.97	1.00													
RY24	-0.28	0.18	0.64	-0.34	0.14	0.75	-0.63	-0.63	-0.47	0.61	0.60	0.56	0.81	0.93	0.91	0.94	0.96	1.00												
CL0	0.64	-0.57	-0.57	0.63	-0.52	-0.58	0.86	0.90	0.60	-0.88	-0.89	-0.83	-0.36	-0.47	-0.40	-0.58	-0.64	-0.64	1.00											
CL2	0.59	-0.53	-0.51	0.57	-0.49	-0.52	0.83	0.87	0.59	-0.86	-0.88	-0.82	-0.35	-0.47	-0.40	-0.56	-0.63	-0.63	0.99	1.00										
CL24	0.57	-0.53	-0.48	0.54	-0.48	-0.48	0.71	0.74	0.40	-0.76	-0.77	-0.71	-0.28	-0.39	-0.33	-0.47	-0.53	-0.53	0.88	0.93	1.00									
CA0	-0.68	0.60	0.39	-0.62	0.65	0.32	-0.65	-0.67	-0.46	0.74	0.74	0.71	0.01	0.07	0.01	0.20	0.24	0.25	-0.80	-0.83	-0.85	1.00								
CA2	-0.66	0.66	0.39	-0.61	0.63	0.32	-0.68	-0.70	-0.48	0.75	0.77	0.74	0.03	0.11	0.04	0.23	0.28	0.28	-0.81	-0.87	-0.88	0.96	1.00							
CA24	-0.67	0.66	0.40	-0.60	0.63	0.35	-0.65	-0.67	-0.44	0.72	0.74	0.70	0.06	0.15	0.09	0.26	0.31	0.31	-0.78	-0.82	-0.89	0.92	0.93	1.00						
CB0	-0.21	0.13	0.59	-0.27	0.10	0.73	-0.52	-0.54	-0.34	0.54	0.52	0.43	0.91	0.90	0.87	0.90	0.91	0.91	-0.63	-0.62	-0.56	0.28	0.29	0.31	1.00					
CB2	-0.21	0.13	0.59	-0.28	0.11	0.75	-0.52	-0.53	-0.35	0.53	0.51	0.40	0.78	0.94	0.88	0.93	0.94	0.91	-0.64	-0.65	-0.59	0.29	0.33	0.35	0.92	1.00				
CB24	-0.20	0.13	0.58	-0.27	0.10	0.74	-0.53	-0.54	-0.36	0.54	0.52	0.42	0.81	0.94	0.88	0.92	0.94	0.92	-0.63	-0.65	-0.60	0.29	0.31	0.35	0.93	0.97	1.00			
CY0	-0.32	0.24	0.64	-0.37	0.19	0.78	-0.62	-0.64	-0.43	0.64	0.62	0.51	0.78	0.91	0.85	0.92	0.95	0.93	-0.74	-0.74	-0.67	0.40	0.42	0.44	0.95	0.97	0.97	1.00		
CY2	-0.27	0.20	0.60	-0.32	0.16	0.74	-0.58	-0.60	-0.39	0.59	0.58	0.48	0.77	0.90	0.84	0.90	0.93	0.91	-0.72	-0.74	-0.68	0.40	0.44	0.45	0.93	0.97	0.97	0.98	1.00	
CY24	-0.28	0.21	0.60	-0.34	0.18	0.74	-0.60	-0.61	-0.41	0.62	0.60	0.50	0.77	0.90	0.84	0.91	0.93	0.91	-0.72	-0.74	-0.71	0.41	0.44	0.46	0.92	0.97	0.99	0.97	0.98	1.00

Colour of meal, flour and noodles

Using a Hunterlab colorimeter, colour was measured on meal, flour, and raw and cooked noodles at 0, 2 and 24 hours after being sheeted or cooked, respectively. Percentage ash, particle size index and polyphenol oxidase activity (PPO) were all found to be correlated with noodle colour.

Percentage ash was significantly correlated in the range of r = 0.4-0.6 with all colour measurements. Ash represents the mineral content of the wheat kernel. L-values (whiteness) were always negatively correlated with ash percentage while a-values (redness) and b-values (yellowness) were positively correlated.

Particle size index (PSI) is a measurement of grain hardness and was positively correlated to L-values for all colour measurements and negatively correlated to all a-value measurements. PSI was also negatively correlated to b-values but only with meal and flour. However, when the product of PSI*ASH was considered, correlations to any of the end-product colour measurements were not significant, supporting the negative correlation observed between ash and PSI.

PPO activity was found to be significantly correlated only with the L-values of raw noodles 24 hours after the noodle had been sheeted. This observation is reported in the literature. The enzyme activity of this group of oxidative enzymes is expected to produce colour changes in raw noodle over time. Fresh noodles provide no time for the enzymes to and cooking will inactivate the enzymes.

Noodle texture

Texture profile analysis was used to characterize the physical characteristics of white salted noodles. Of the characteristics recorded, hardness, springiness and chewiness showed significant correlations to flour protein, flour a-value, mixograph peak height, bandwidth energy and total energy, farinograph flour water absorption, raw and cooked noodle L and a-values.

Mapping

Development of the molecular map has not progressed as hoped due to inadequate funding and loss of personnel. As a result, no new mapping results are available at this time.

Seventy-three loci have been mapped on the AC Karma/SC8021V2 population. The loci are distributed on all 21 chromosomes, ranging from one marker per chromosome to as high as eight. Early marker efforts have been focused on groups 1 and group 3 chromosomes because regions of these have been implicated in preharvest sprouting resistance, which is the other reason why this population is being studied.

Mapping analysis was conducted by W. Cao and is summarized in Table 6. The combined data resulted in a number of observations:

1. The effect of grain hardness on many of the traits was demonstrated by the high frequency that the chromosome 5D locus was detected as being significant.
2. Significant loci were detected for several noodle colour and quality parameters. Raw noodle colour for both L and B values measured at 24 hours after the noodles were made was

detected with the same marker (WMC25E02) that detected a yellow colour effect (B-value) on cooked noodles at 0 hours after they were made.

3. A marker for viscosity breakdown was detected both with 1999 and the combined data set. Markers that are significant in each year as well as in combined datasets will be viewed as more robust.
4. Loci that were significantly associated with mixograph parameters were observed. This was not the case for farinograph parameters with the exception of flour absorption (FAB) that is highly dependent on starch damage, which is associated with grain hardness.

Table 6. Summary of where data was collected, analysis of variance F-statistic P-values for each trait, and results of preliminary mapping analysis

	Mapping analysis[b]					
	1999			1999-2000		
Character[a]	Marker	Chromosome	Effect	Marker	Chromosome	Effect
Agronomic and disease traits						
YLD						
TWT				WMC25E02	4D	2.33
KWT						
HT	WMS120	2B	-7.05			
				WMC25E02	4D	13.5
MAT	WMS484	2D	3.72			
LD BT	WMS164, 469	1A; 6D	-18.29; 16.49			
Quality traits						
PRO_LECO						
GPRO_NIR	WMS190	5D	-1.42	WMS190	5D	9.42
PSI	WMS190	5D	5.76	WMS190	5D	9.42
SKCS				WMS190	5D	-34.87
PPO						
SDS				WMS190	5D	11.8
FN						
MEAL_L	WMS190	5D	1.53	WMS190	5D	2.34
MEAL_A	WMS190	5D	-0.23	WMS190	5D	-1.36
MEAL_B	WMS190	5D	-0.66	WMS190	5D	-4.1
FYLD_PCT				WMS190	5D	-4.1
FPRO_LECO				WMC25E02	4D	0.41
FLOUR_L	WMS190	5D	1.33	WMS190	5D	2.02
FLOUR_A	WMS190	5D	-0.16	WMS190	5D	-0.29
FLOUR_B				WMS190	5D	-2.11
MDT						
PKH				WMS190	5D	-3.86
SAP				WMC23A02	1D	-1.32
BWE				WMS190	5D	-3.12

Table 6. Summary of where data was collected, analysis of variance F-statistic P-values for each trait, and results of preliminary mapping analysis

	Mapping analysis[b]					
	1999			1999-2000		
Character[a]	Marker	Chromosome	Effect	Marker	Chromosome	Effect
BW_AT_6						
TEG				GWM273	1B	-18.68
ASH						
FAB	WMS190		-2.03	WMS190	5D	-2.29
DDT						
STA						
MTI	WMS111	2B;7DS	-10.99	WMS190	5D	12.05
PEAK_VIS				WMS337 WMC24C03	1D	-68.66
V_AT_START				WMC24C03	1D	-57.01
V_AT_EDN				WMC24C03	1D	-57.32
BREAKDOWN	WMS337;382a	1D; 2A,2B,2D	-22.2	WMS337 WMC24C03	1D	-20.65
SETBACK				WMC24C03	1D	-33.93
LV						
RL0						
RL2						
RL24	WMS608b	2D; 4D	-2.75			
RA0				Hardness	5D	-0.39
RA2						
RA24				WMC25E02	4D	-0.39
RB0						
RB2						
RB24						
RY0						
RY2						
RY24				WMC25E02	4D	-4.01
CL0						
CL2						
CL24						
CA0				WMS190	5D	-0.25
CA2						
CA24						
CB0						
CB2						
CB24						
CY0				WMC25E02	4D	-3.95
CY2						
CY24						
HARDNESS						
COHESIVE				WMS357	1A	0.77

Table 6. Summary of where data was collected, analysis of variance F-statistic P-values for each trait, and results of preliminary mapping analysis

	Mapping analysis[b]					
	1999			1999-2000		
Character[a]	Marker	Chromosome	Effect	Marker	Chromosome	Effect
ADHESIVE						
AD_FORCE						
SPRING						
STRING						
GUMMY				WMS357	1A	18.15
CHEWY				WMC25B12	4A	0.37

[a]Character definitions are found in Appendix C.

[b]Markers are wheat microsatellites (WMS). Chromosome indicates the genomic location of the marker and thus the trait. The effect is shown in the units the trait was measured in. A positive effect indicates that presence of the marker is associated with increasing the trait value. More than one marker can be associated with a trait and one trait may be controlled by genes at more than one location in the genome.

References

American Association of Cereal Chemists. 1995. Approved Methods of the AACC. 9th Edition. The Association, St. Paul, MN.

Morris, C.F., Anderson, J.V., Bettge A.D. and Correll, M.E. 1998. Distribution of PPO activity among a large number of hexaploid wheat genotypes using an improved L-DOPA assay. Cereal Foods World 43:518.

Prospects

Recently, a Matching Investment Initiative project was given support. As part of this project, a map of the AC Karma/SC8021V2 population with 200+ markers will be developed within the next two years. This map can then be used to look for genetic associations to the quality traits measured in this project. Thus the eventual goal of this project will be achieved. Another similar study has been underway the Cereal Research Centre involving another population with a similar type of quality data set. This population will also be mapped. Together, these two studies will provide an excellent opportunity to identify genetic markers that explain variation for various wheat quality traits and the opportunity for effective indirect selection, early in breeding programs, through the use of DNA markers rather than traditional wheat quality tests.

Additionally, new predictive tests underdevelopment at the Cereal Research Centre will be compared to the traditional tests used in this project using the residual seed and flour from this project. These predictive tests include a starch swelling test, glutenin fraction estimation using spectrophotometry (Odean Lukow) and ultrasound (Miklos Kovacs).

All of the residual seed and flour produced in this project has been moved to the CRC in Winnipeg for longer term storage. I believe I will be able to utilize this material in future projects allowing for multi-location experiments to be considered without having to grow these materials again.

Personnel

List of cooperators

The number of people participating in this project has grown and illustrates the value of this project.

Stephen Fox – AAFC-CRC (formerly U of S): project leader
Connie Perron – U of S: coordinator of quality trait evaluation
Ron Knox – AAFC-SPARC: site Swift Current cooperator
Ron DePauw – AAFC-SPARC: site cooperator at Swift Current and originator of the population under study
Doug Brown – AAFC-CRC: Glenlea site cooperator
Ken Armstrong – AAFC-ECORC – genetic mapping
Wen Cao – AAFC-ECORC – genetic mapping
Judith Fregeau-Reed – AAFC-ECORC – kernel hardness evaluation
Dr. Dave DeKoeyer is no longer participating in this project, as he has taken on other responsibilities in eastern Canada.

Summer students

Sherry Smith, a third year coop student, was hired for the period May to September, 2000 to manage and conduct quality data collection. She is a coop student at the U of S and uses the experience she gained last year towards getting data collected and entered into files. Two additional summer students were hired to assist her.

Three summer students were hired to collect the quality data during 2001: Kendall Avis, Carolyn Cricket, and Theodosia Stadnyck. All three students are undergraduates at the University of Saskatchewan. We expect Kendall Avis to return for summer 2002 and to hire two more students.

Three summer students were hired to collect the quality data during 2002: Kendall Avis, Naomi Carriere, and Senait Ghebre.

Equipment

None

Project Developed Materials

None

Project Photographs

None

Acknowledgements

Connie Perron who managed the collection of the quality data throughout the project.
Technical staff of the durum/CPS breeding group is thanked for managing the seed supplies that are the basis for this project.

AAFC-CRC for providing supplementary funding to allow completion of data collection in 2002.

Expense Statement

A statement is provided by University of Saskatchewan financial services department.